Ultracytochemical Studies of Lysosomal Function in the Macrophages of Human Leprosy—I.[1]

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In human leprosy there is a wide spectrum of clinical disease ranging from lepromatous leprosy (LL) at one end to tuberculoid leprosy (TT) at the other ([9]). The clinical spectrum of human leprosy seems to depend on individual differences in the cell-mediated immune (CMI) response against *Mycobacterium leprae* ([12]). The basis for this variation is not known.

Macrophage functions and their immune mechanisms play a very important role in leprosy throughout the spectrum. It was, therefore, thought useful to study the lysosomal status and function in the macrophages of all types of leprosy.

Lysosomes are subcellular organelles and are characterized by their polymorphism ([2]). They cannot easily be distinguished by any typical size or structure. Only their biochemical properties, i.e., principally their enzyme content, and the structure-linked latency of these enzymes define them individually. The ultracytochemical technique, using acid phosphatase as the marker enzyme, seems to be the one most suitable for the study of lysosomes because it enables them to be studied utilizing their biochemical activity (hydrolytic enzyme activity) without destroying the cell membrane structure.

Lysosomes take part in the intracellular digestion phenomena involving both exogenous materials taken up by the cell (heterophagic function) and cellular components (autophagic function). The intracellular functions of lysosomes depend on their lytic potential; these organelles are involved both in physiological and pathological processes ([3]).

The present study aims to show lysosomal status and their functional behavior in the macrophages across the leprosy spectrum, including the reactional states. Such information could be of relevance in understanding the microbicidal killing mechanism as well as the immunopathology of the disease process.

MATERIALS AND METHODS

The material for ultracytochemical lysosomal study consisted of skin biopsies from normal healthy individuals and untreated cases across the leprosy spectrum: Normal skin, 4; tuberculoid (TT), 7; borderline tuberculoid (BT), 4; borderline tuberculoid with reactions [BT(R)], 4; lepromatous (LL), 12; and lepromatous with reactions [LL(R)], 4.

At least two biopsies were taken from each case. Biopsies were from different lesions in the BT, BB, BL, and LL cases, and from different sites in the same lesion in TT cases. The biopsy specimen was cut into two 1 × 1 mm pieces for fixation. One piece was immediately fixed in 2% purified glutaraldehyde in 0.1 M sodium cacodylate-HCl buffer (pH 7.2) containing 8% sucrose for 1 hr at 4°C, and then washed for 1 hr at 4°C in a 0.25 M sucrose containing 0.1 M buffer (sodium cacodylate-HCl, pH 7.2). This protocol is based on earlier reported work ([1, 7, 10]). The other portion of the biopsy material was fixed in 10% neutral Formalin for histological studies (H&E staining) and Ziehl-Neelsen (ZN) staining to demonstrate acid-fast bacilli.

The ultracytochemical technique for the demonstration of lysosomes used acid phosphatase as the marker enzyme ([5]). Sodium-beta-glycerophosphate was used as the

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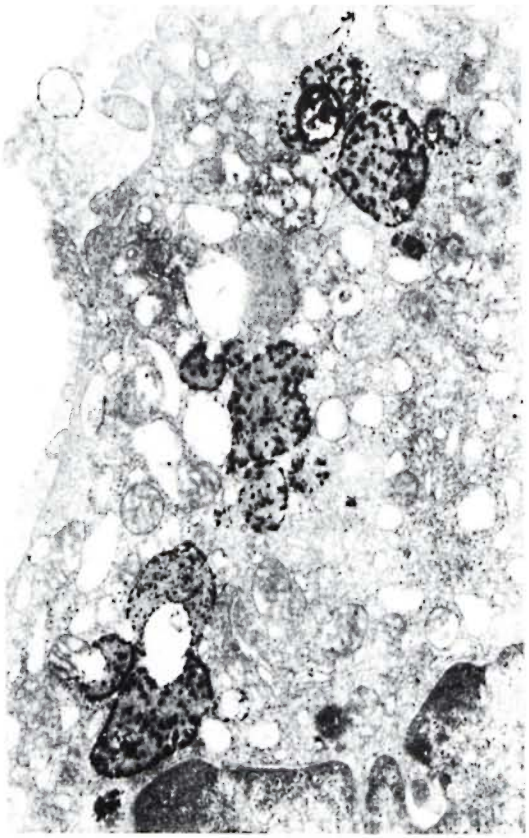

FIG. 1. Electronmicrograph showing lysosomes in macrophages of normal skin. Ultracytochemical demonstration shows well-preserved lysosomal morphology and membrane integrity (Gomori acid phosphatase technique ×20,000).

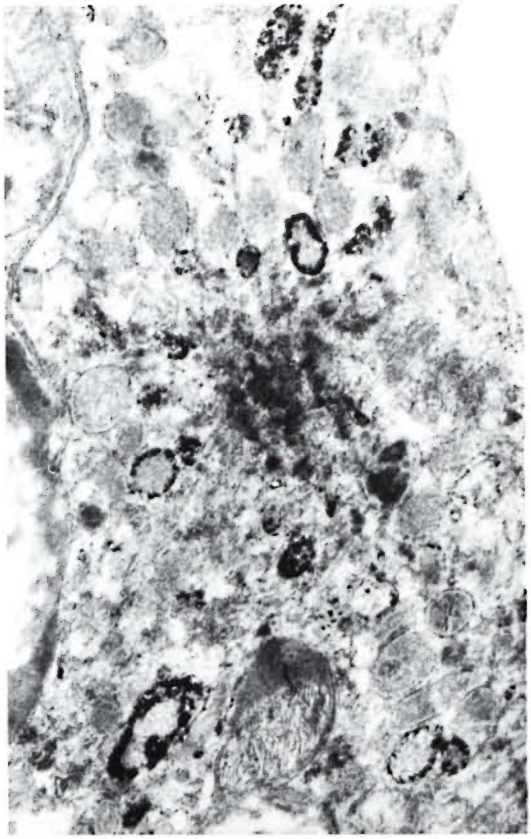

FIG. 2. Electronmicrograph showing lysosomes in macrophages of tuberculoid (TT) leprosy with well-preserved lysosomal morphology and membrane integrity (Gomori acid phosphatase technique ×13,000).

substrate. The incubation mixture was prepared in 0.05 M acetate buffer in which lead nitrate [$Pb(NO_3)_2$] and sucrose were also dissolved. Tissue sections, 40–50 μm thick after fixation in 2% glutaraldehyde in 0.1 M sodium cacodylate-HCl buffer (pH 7.2) containing 8% sucrose, were incubated in this mixture for 90 min at 37°C ([5, 7]). This was followed by osmification, dehydration, and embedding in Spurr resin ([11]) at 70°C for 8–12 hr. After ultrathin sectioning and uranyl acetate treatment, observations were carried out using an electron microscope.

Several macrophages from each case were screened under the electron microscope and electronmicrographs were taken. The lysosomes appeared to be of different shapes and sizes due to their characteristic nature of polymorphism. They showed black granular precipitate (dense or feeble).

RESULTS

Lysosomes in the macrophages of normal skin (Fig. 1), TT (Fig. 2), BT and BT(R) (Fig. 3) showed that a limiting membrane was intact and that lysosomal morphology was well preserved with demonstrable ultracytochemical acid phosphatase activity.

The number of lysosomes in TT, BT and BT(R) macrophages was increased when compared to the macrophages of normal skin. However, this increase in lysosomal number was more pronounced and significant in BT(R) (Fig. 3). Such changes were observed in almost all of the macrophages examined.

In some of the macrophages of active LL cases, lysosomal morphology and its limiting membrane integrity were lost. However, such changes were predominantly present in the macrophages from LL(R) cases

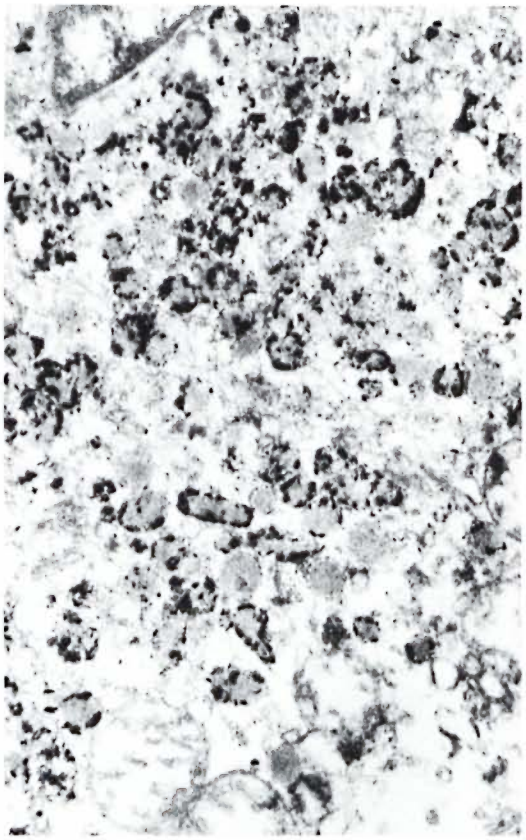

FIG. 3. Electronmicrograph showing lysosomes in macrophages of BT(R) with well-preserved lysosomal morphology and membrane integrity. There is a pronounced and significant increase in the number of lysosomes (Gomori acid phosphatase technique ×13,000).

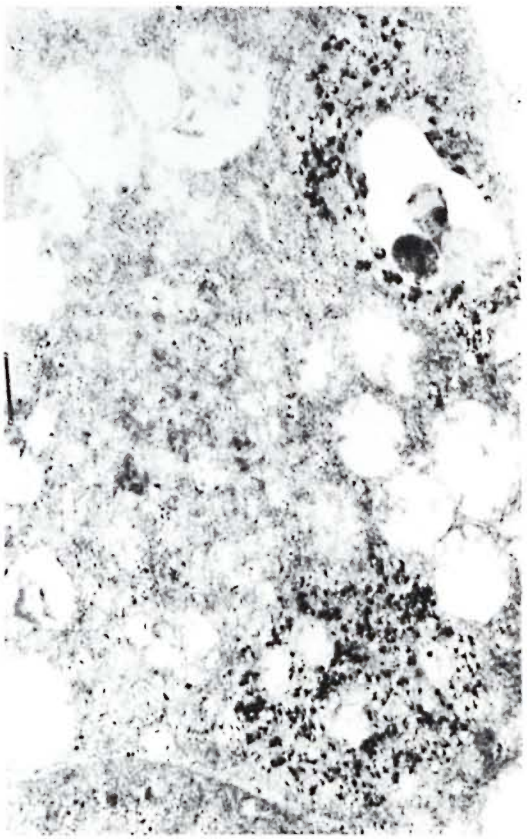

FIG. 4. Electronmicrograph showing lysosomes in macrophages of LL(R). Lysosomal morphology is lost, the lysosomal cell membrane is ruptured, acid phosphatase reaction product is spilled over and wrapping around *M. leprae* present in the transparent zone (Gomori acid phosphatase technique ×16,000).

(Fig. 4). The lysosomal membrane rupture resulted in the spill-over of the enzymatic reaction product. It was observed that the ruptured lysosomes (acid phosphatase reaction product) encircled the *M. leprae* present in the electron-transparent zone in the macrophages (Fig. 4). Also, the spill-over acid phosphatase reaction product was seen scattered all over the macrophages.

DISCUSSION

Lysosomal morphology and its membrane status were studied in the present ultracytochemical work using acid phosphatase as the marker enzyme. Lysosomes in normal skin, TT, BT, and BT(R) showed that the limiting membrane was intact. Lysosomal morphology was well preserved with demonstrable cytochemical acid phosphatase activity. However, there was a slight increase in lysosomal number in TT and BT cases, but it was more pronounced and significant in the BT(R) cases.

In LL and LL(R) cases, lysosomal membrane integrity was invariably lost, and the membrane rupture resulted in the spill-over of enzymatic product. It was observed that lysosomes encircled the *M. leprae,* as seen in the electron-transparent zone. However, this lysosomal wrapping mechanism (LWM) ([8]) did not result in the lysis of *M. leprae.* On the other hand, this is speculative since it is difficult to know the sequence of events of an individual infected cell. Instead, standard phagosome-lysosome fusion may be responsible for these ultrastructural changes. More extensive and planned studies are necessary to unravel this mechanism.

CMI in LL and LL(R) is almost always absent, while in the tuberculoid forms of leprosy CMI is quite strong and positive. The macrophages, which play an important role in the "affector" and "effector" limbs of the immune response, could be a significant contributing factor in the two types of leprosy, i.e., tuberculoid and lepromatous. Job (6) emphasized that the lysosomal response and possible impact on leprosy bacilli in macrophages still had to be investigated, because these cells were the principal "host" in both tuberculoid and lepromatous forms of the disease.

Lysosomal wrapping mechanism (LWM) as inferred in our present study signifies two important observations: a) It is quite evident that lysosomes encircle the *M. leprae* present in the electron-transparent zone but are not able to kill and lyse the *M. leprae.* b) It appears that the electron-transparent zone, which is probably lipid in nature, acts as a barrier and the lysosomes are unable to directly interact with the *M. leprae* present in this zone, which corroborates the findings of Draper and Rees ([4]).

It also appears that the macrophages in the tuberculoid form are activated, while they are in a passive form in lepromatous leprosy and its reactional states. The loss of integrity of lysosomal membranes was observed in most of the active LL cases and in all of the LL(R) cases studied. It is not an artifact or due to technical faults, since such changes were not observed in biopsies from other types of leprosy cases studied in the present work. Such changes can easily be explained in reactional states but why they should occur in some of the active LL cases without reaction is not clear. Lysosomal function is directly related to the status of macrophages which is discerned in terms of CMI.

The increased serum levels of lysosomal enzymes in reaction and in active LL cases, as observed in our previous biochemical study ([13]), may thus be explained since there is a spill-over of enzyme when the limiting lysosomal membrane ruptures and loses its integrity. This explanation is presently tentative, and future studies are planned to explain the phenomena and to provide sufficient evidence to prove that stabilization of lysosomal membranes takes place when a phase of subsidence is achieved by using drugs such as corticosteroids and chloroquine.

The ultracytochemical study of lysosomal enzymes thus has two distinct advantages over the biochemical methods: a) The intracellular location of an enzyme may be determined if the end product is precipitated before it diffuses away from its site of production. b) The proportion of the total activity manifest under normal conditions may be determined. The latent activity has been shown to be an informative index of pathological or physiological changes in the lysosomes and, further, it is possible to visualize the demonstration and localization of the enzymes directly in the lysosomes present in a particular cell type.

The importance of the present ultracytochemical study of lysosomes is that it may help in correlating the lysosomal enzyme activity, lysosomal morphology and its limiting membrane integrity with the waxing and waning immune response throughout the leprosy spectrum.

SUMMARY

The present study was undertaken to understand the lysosomal status and function in macrophages across the leprosy spectrum, including the reactional states. Acid phosphatase was used as the marker enzyme to demonstrate lysosomes at the electron microscopic level. It was observed that lysosomal morphology was well maintained in the macrophages of tuberculoid, borderline tuberculoid, and borderline tuberculoid leprosy in reaction, while they lost their cellular morphology and cell membrane integrity in lepromatous leprosy and lepromatous leprosy in reaction. The importance of these findings is discussed.

RESUMEN

El presente trabajo se hizo con la idea de entender el estado y la función lisosomal en los macrófagos a lo largo del espectro de la lepra incluyendo los estados reaccionales. Se usó la fosfatasa ácida como marcador enzimático para visualizar los lisosomas a nivel de la microscopía electrónica. Se observó que la morfología lisosomal estuvo bien preservada en los macrófagos de los pacientes con lepra tuberculoide, tuberculoide-intermedia, y tuberculoide-intermedia en reacción, mientras que perdieron su morfología celular y su integridad de membrana en los casos de lepra lepro-

matosa reaccional. Se discute la importancia de estos hallazgos.

RÉSUMÉ

Cette étude a été entreprise dans le but de comprendre le rôle des lysosomes et la fonction des macrophages, dans les différents types de lèpre tout au long du spectre de la maladie, y compris dans les états réactionnels. On a utilisé la phosphatase acide comme enzyme marqueur pour mettre en évidence les lysosomes par microscopie électronique. On a observé que la morphologie des lysosomes était bien conservée dans les macrophages, chez les sujets atteints de lèpre tuberculoïde, de la forme tuberculoïde dimorphe de la maladie, et dans la forme dimorphe tuberculoïde en réaction. Par contre, ils perdaient leur morphologie cellulaire, et l'intégrité de la membrane cellulaire était détruite, dans la lèpre lépromateuse, ainsi que dans la lèpre lépromateuse en réaction. On discute l'importance de ces observations.

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REFERENCES

1. Brunk, T. U. and Ericsson, J. L. E. The demonstration of acid phosphatase in *in vitro* cultured tissue cells; studies on the significance of fixation, toxicity and permeability. Histochem. J. **4** (1972) 349–363.
2. De Duve, C. Lysosomes, a new group of cytoplasmic particles. In: *Subcellular Particles.* Hayashi, T., ed. New York: The Ronald Press Co., 1959, p. 128.
3. De Duve, C. and Wattiaux, R. Functions of lysosomes. Ann. Rev. Physiol. **28** (1966) 435–492. (330 ref.)
4. Draper, P. and Rees, R. J. W. Electron transparent zone of mycobacteria may be a defence mechanism. Nature **228** (1970) 860–861.
5. Gomori, G. *Microscopic Histochemistry.* Chicago: University of Chicago Press, 1952, p. 189.
6. Job, C. K. Lysosomal activity of macrophages in leprosy. Arch. Pathol. **90** (1970) 547–552.
7. Mayahara, H. and Chang, J. P. Electron microscopic study of phosphatase activity in cultured human cystic fibrosis fibroblasts. Acta Histochem. Cytochem. **11** (1978) 449–459.
8. Mayahara, H. and Ogawa, K. Lysosomal wrapping mechanism observed in the autophagolysosome formation. In: *Histochemistry and Cytochemistry.* Takeuchi, T., Ogawa, K. and Fujite, S., eds. Kyoto: Japan Society of Histochemistry and Cytochemistry, 1971, pp. 29–30.
9. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity; a five-group system. Int. J. Lepr. **34** (1966) 255–273.
10. Sabatini, D. D., Bensch, K. and Barrnett, R. J. Cytochemistry and electron microscopy: the preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol. **17** (1963) 19–58.
11. Spurr, A. R. A low-viscosity, epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26** (1969) 31–43.
12. Turk, J. L. and Bryceson, A. D. M. Immunological phenomena in leprosy and related diseases. Adv. Immunol. **13** (1971) 209–266.
13. Venkatesan, K., Bharadwaj, V. P., Ramu, G. and Desikan, K. V. Serum beta-glucoronidase in leprosy—a preliminary report. Indian J. Med. Res. **68** (1979) 553–556.